**Cellular Biophysics**
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**Indian Institute of Science Education and Research, Pune**
**Lecture 55**
**Membrane Deformation**

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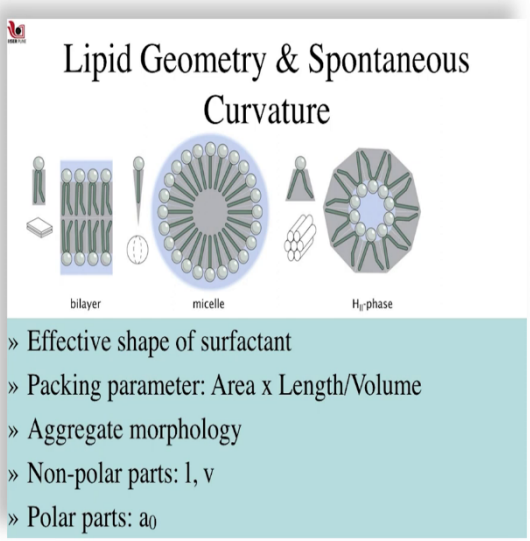


Hi, welcome back. So last time we spoke about lipid geometry and spontaneous curvature, and we had discussed the various shapes of the lipids that can give rise to different shapes of membranes. In other words, how the molecular component shapes, geometry can affect the microscopic lipid assembly.

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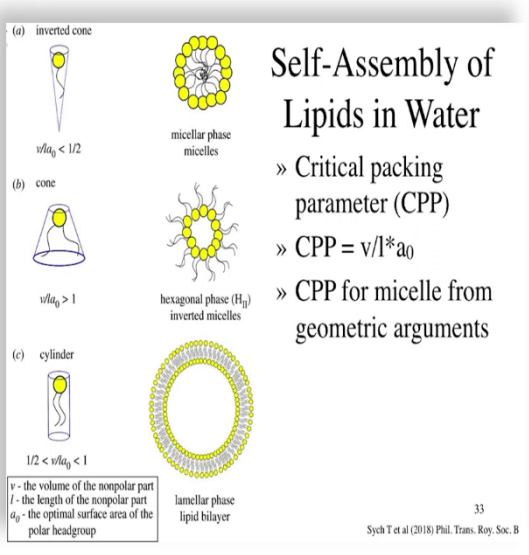

And we talked about so-called critical parameter, packing parameter, which we defined as

v/(l*a0) , and l is the tail length, the hydrophobic tail, a0 is the area of the surface head group, and v is the volume of the tail. In such a case, we said and we actually tried to demonstrate this, that micellar phases, hexagonal phases, and other phases, in fact we only did this for micellar phases, can be shown geometrically to fall within a certain range.

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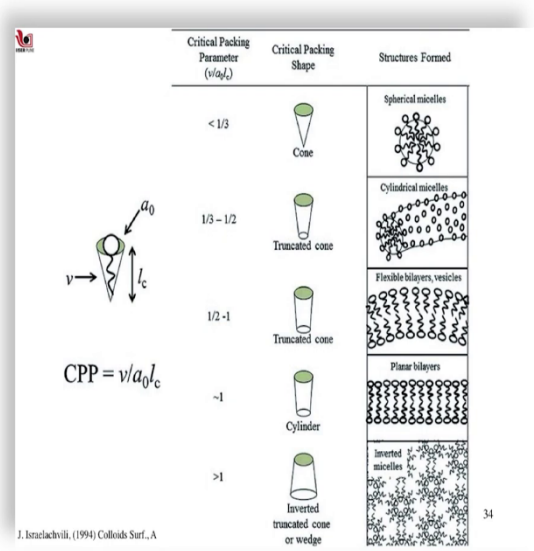


So, a conical lipid shape with a critical parameter, packing parameter of less than 1 by 3, less than or equal to, I think we said, will form spherical micelles, and a similar range of values between one-thirds and half will give, with a truncated cone, will give you cylindrical micelles, half to one will give you a truncated cone flexible bilayers, vesicles, approximately 1 will give you a planar bilayer.

So in other words, if you have cylinders you will have cylindrical structures that nicely pack into, are more favoured to form just simply a flat layer, and inverted truncated cones or wedges from these kind of inverted micells, which I think we call so called hexagonal phases, and you kind of see that in how they are arranged with each other, with the hydrophobic tails outside, and forming little cavities inside of the hydrophilic parts.

This is all theory due to Israel actually, 1999. So this is what we talked about previously along with membrane models, and the mobility of membrane components which we discussed in terms of some experimental methodologies which, some of, which are going to deal with later when an expert comes to talk about FCS. FRAP has I think been part of the

experimental techniques I have already uploaded as a technique presentation write-up slide, and we will discuss it some other time, not for today.

So, for today, I want to talk to you about membrane shape change and curvature, GUVs and lipid compositions, deformation mechanics, and how to measure the mechanical properties of membranes using micro pipette aspiration for membrane stiffness measurements. And the last part, I think has been dealt very briefly by Kia already, so it is going to be a bit of a revision.

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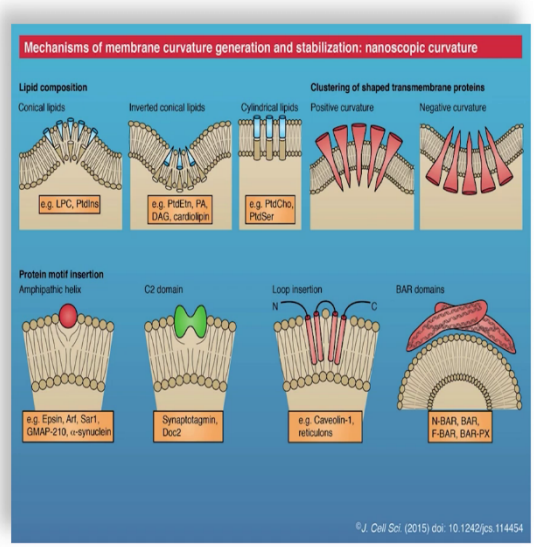


So, let us get straight to it with regard to membrane shape change and curvature. We can see that in experimental systems and cells in fact nanoscopic curvature can be generated by lipid composition or protein motif insertion. So, you see in the top row, the lipid composition that determines the curvature induction by these kinds of conical lipids being inserted in a certain specific way, causing either positive or negative curvatures.

Cylindrical lipids on the other hand will then pin it and create a so-called straight line with non-curved structures or clustering of transmembrane proteins with either positive or negative curvature, all this is with respect to the inside of the cell. Protein motif insertion in that sense, with either amphipathic helices or these kind of c2 domains, loop insertions, bar domains can also cause curvature.

These are very important physiologically and some of you may have heard the talks of Thomas or other people on endocytosis like Nagaraj, and these may be familiar to you. But this is nanoscopic. In other words, if you remember we talked about order of magnitude estimates and we say that proteins are of a few nanometres in size, so a few proteins will still

be a few nanometres, maybe tens of nanometres, so your curvature is over tens of nanometres in size. So this is important to bear in mind.

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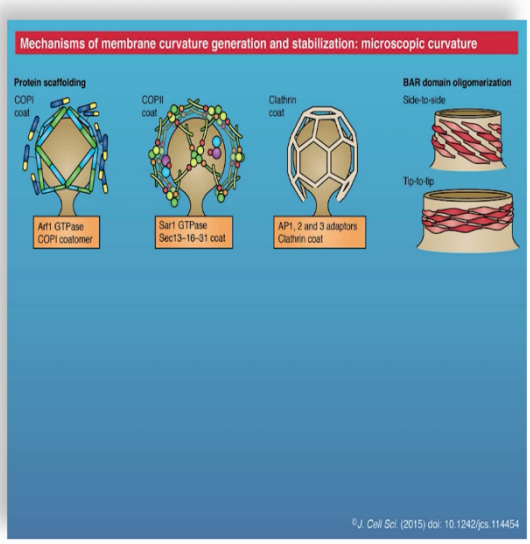


Because in contrast to microscopic curvature, where you can actually see a micrometre scale. And these are protein scaffolding structures like copon codes, copper coats, clathrin coats, these are very important for basic endocytosis budding, and bar domain proteins, cytoside arrangement is seen, or tip-to-tip arrangement is seen to be one of the many ways by which these kind of microscopic, micron-sized curvature structures can be formed. These are vital for cell physiology.

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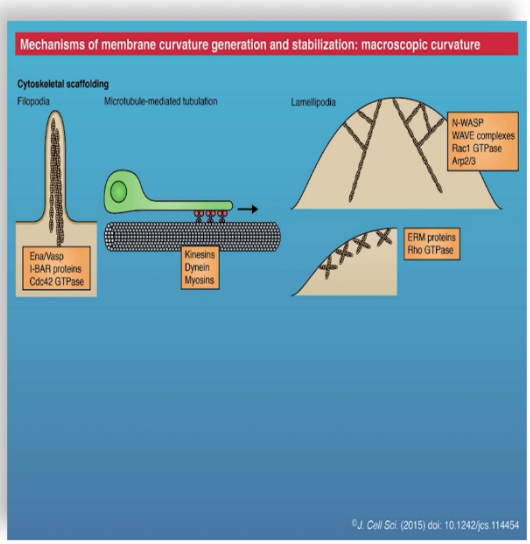


And finally, coming to the whole cell, what we are now referring to as microscopic, that is,, tens of micrometres ranges of curvature, can be seen in the formation of Filopodia, microtubule mediated tubulation, and lamellipodia, are driven by either actin or microtubules.

And these have a lot of bases in biochemistry and this paper that I have referred to here is a nice review that you are welcome to go back and look at in journal of Cell Science from 2015. But all this is fine, because understanding the mechanics of these membranes requires a more purified system, a more easily manipulated, easily measured system.

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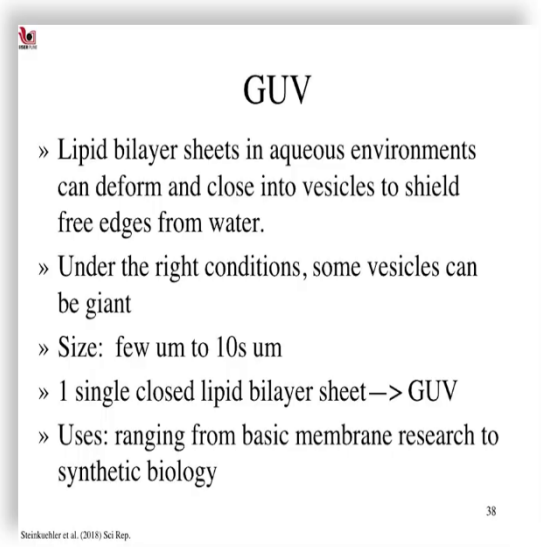


And for that, giant unilateral vesicles have proven to be a god centre, I am sorry, I should not do such words, have proven to be rather serenditiously useful. These in fact consist of lipid bilayer sheets and aqueous environments, that can deform and close into vesicles to shield the free edges from water.

Under the right condition some vesicles can be giant. Giant means what? A few micrometres to tens of micrometres, so giant in the cellular context. One single lipid bilayer sheet, therefore that is defined as a giant uni laminar vesicle where it is of micrometre scale in diameter. The uses range from indeed basic membrane research to synthetic biology.

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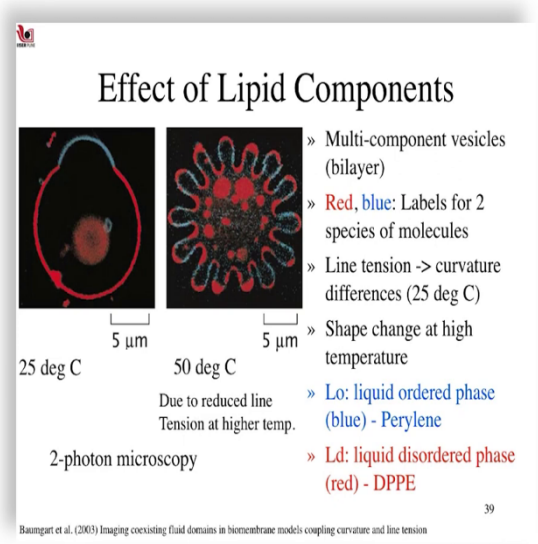


Indeed in GUV work, it has been found strangely that not just does the GUV form these structures, but if you have more than one kind of lipid driving it, so you remember we talked about geometry determining membrane structure, if you, they assumed that we had only one kind of lipid in it. If you have more than one kind, then as these two colours, blue indicating liquid order, blue liquid ordered phase lipid perylene, and disordered phase formed by DPPE, can form indeed structures that self-segregate.

In other words they, you can say, even phase separate, and this separation is also dependent on the ambient temperature. As you see on the left hand side, at 25° Celsius it forms this sort of vesicle with a with a blob to it, it almost resembles a saccharomyces budding cell whereas at 50° Celsius, at a higher temperature, line tension changes cause this kind of almost fingering like structure.

These kind of shape changes are very interesting, because they tell us that while on the one hand from our reductions, from our, I am sorry, from a cell biological inference, you need intracellular mechanics, you need signalling to form structures, but this is telling you that lipid itself, that lipid composition itself has amazing wide dynamics, which can actually spontaneously form these kinds of shapes.

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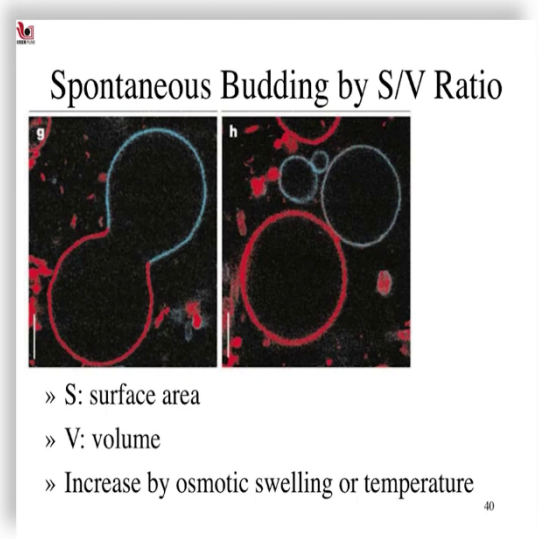


Indeed, the spontaneous budding by modifying the subset at a volume ratio by osmotic swelling or temperatures used frequently to in fact generate giant tuning laminar vesicles of a desired size and shape. So, that is as far as GUVs go. We will return to them in a bit, before which I want to, because we need to discuss what are these mechanical properties that we are going to be discussing.

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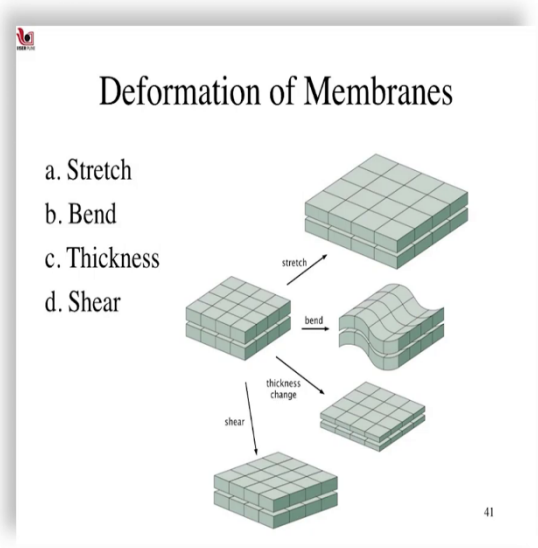


So, the mechanical properties relate to the deformation of membranes and these deformations are in terms of either stretch deformation, so you see the stretching of the membrane which sort of goes parallel to its surface area, it is increased in area in fact, bending which relates to creating curvature, either uniform curvature, meaning one curvature or multiple complex

curvatures, change in thickness which is basically making the membrane compress, meaning to say the leaflets come closer or further apart, so it can be either increase or decrease, and shear which is angular deformation, so let us get to it.

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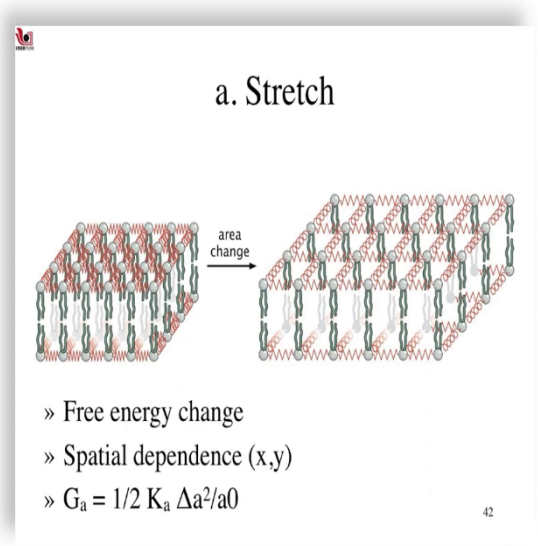


So, in case of stretch, the expression for the free energy of deformation is the free energy Ga of areas deformation,

$$G_a = \frac{1}{2} K_a \Delta \frac{a^2}{a} 0$$

half $K_a$ times $\Delta\ a^2$ upon a0, where $\Delta$ a is the change in area, a0 is the initial area, and G stretch is the energy of stretching. So, in such a case the area stretch modulus $K_a$ is the important parameter to bear in mind. Or in other words, it is what determines the dynamics of or rather the extent to which the membrane can be stretched or the stiffness of the membrane effectively. Please note that this is kind of analogous to your spring expression.

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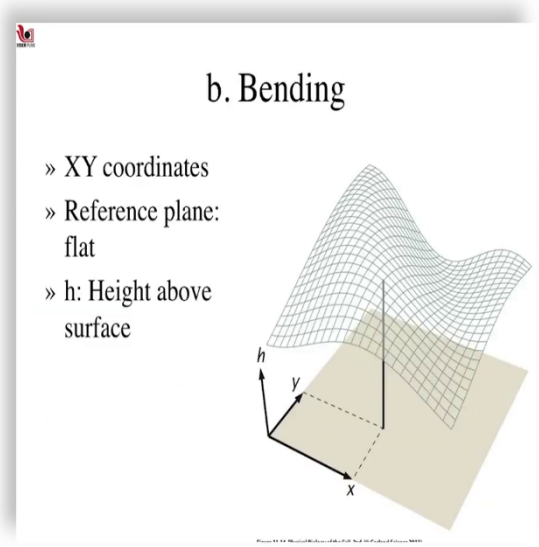


Bending on the other hand is a little more complex, because if you have x y coordinates, and you have a reference plane then you can measure it in terms of a height above a surface.

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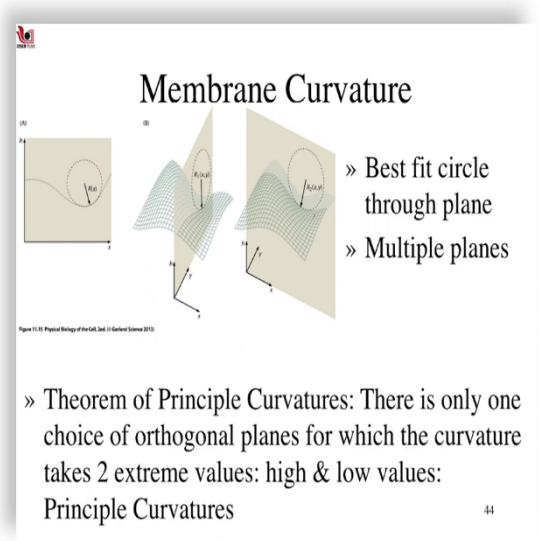


And if you have a complex bend or curvature, then you need to take multiple planes, and find the curvatures along each of those planes. And the best fit circle through that plane gives you the curvature in that plane. And you have the multi planes, and there is a theorem of principle of curvatures which tells you that there is only one choice of orthogonal planes for which the curvature takes two extreme values, the highest, and the lowest. And these are then referred to as the principal curvatures.

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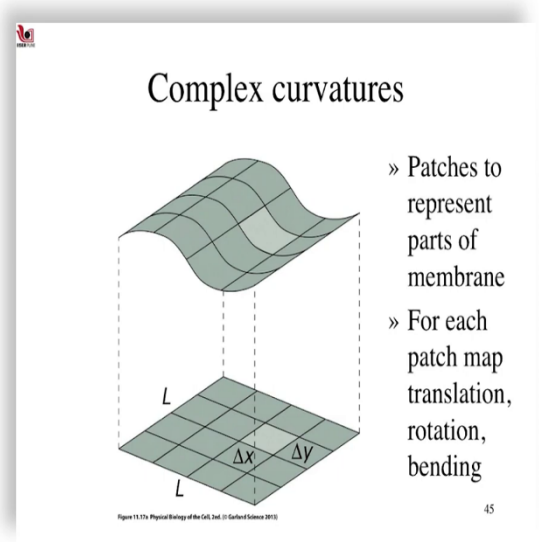


And using this, and the idea that you can break the membrane into patches, you can actually find what is the deformation of the membrane in such a case. The energy of bending is indeed then half $K/R^2$, R being the radius of curvature, and K being the bending modulus, which is which is formulated in terms of the thickness, and the area stretch modulus of the membrane.

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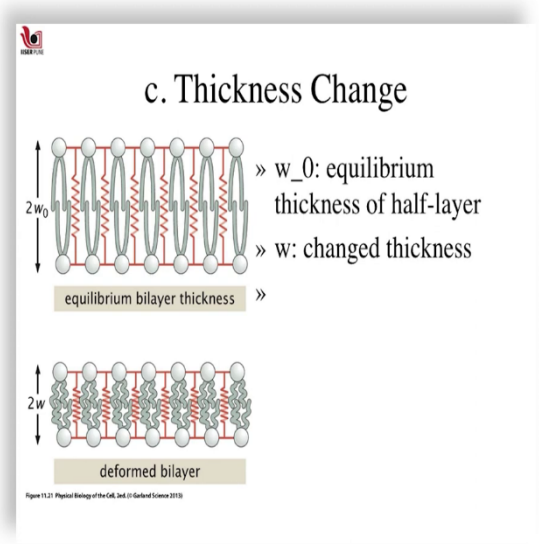


Indeed, thickness change itself is defined, then in terms of the change in the width of the membrane, w naught is equilibrium thickness, and w is the changed thickness, and you can write the energy in terms of half kappa, and then the integral over the squared difference between the current thickness to the initial thickness divided by the initial thickness or the so-called equilibrium thickness.

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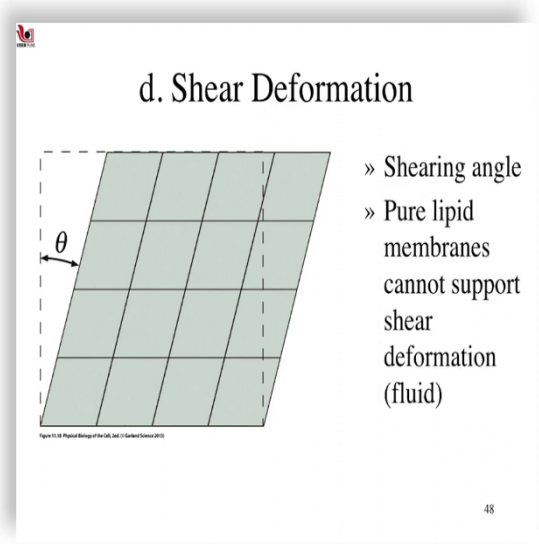


Shear deformation is then just simply quantified in terms of the angle.

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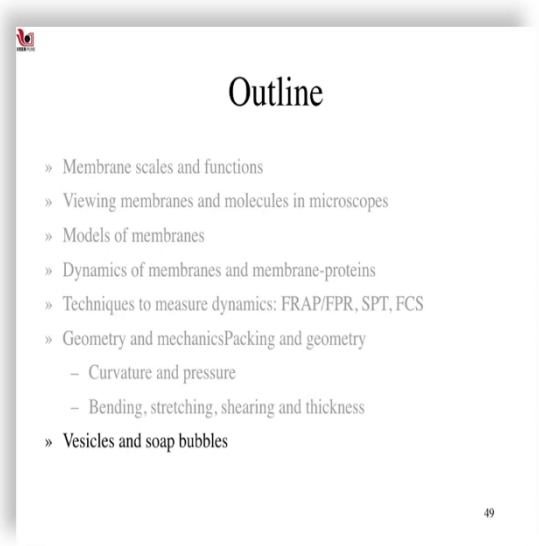


So, given all this, we can say that we have now some ideas at least of what we need to know in terms of what the mechanical deformations of membranes are, but can we measure them? And to do that, we take refuge in very simple theory that is almost 200 years, 150, 200 years old, and this is exactly what care talked to you about that comes from vesicles and soap bubbles.

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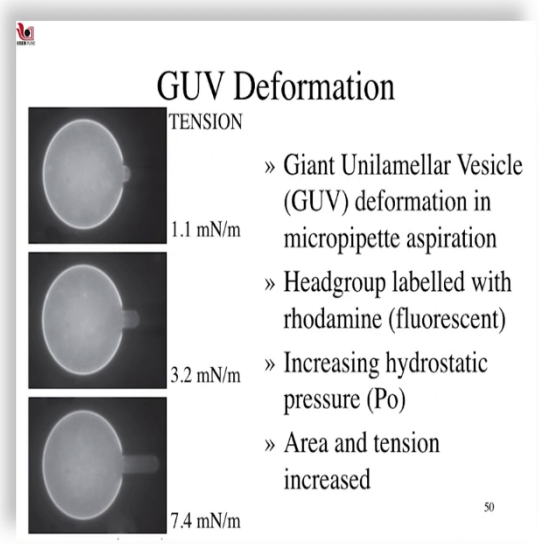


So, Giant Unilamellar Vesicles, which I just mentioned earlier, which are bilayers of micrometre scale, prove to be very good model systems for this kind of measurement. So, GUV deformation can be achieved by creating tension of different values. So the tension values over here are 1.1 milli Newton per meter, 3.2 million per Newton meter, and some point 3 milli Newton per meter, and the experiment here involves visualizing the lipid by head group labelling the laboured lipid using rhodamine, and increasing the hydrostatic pressure, and measuring the area and tension, as the tension is increased.

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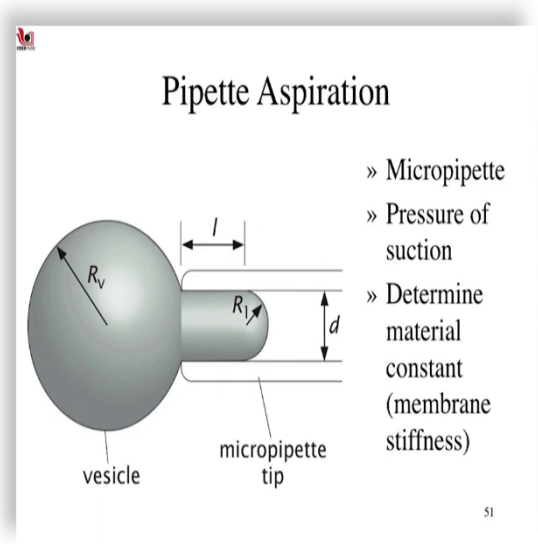


So, this pipette aspiration or micropipette, aspiration experiment is set up in the following way, that, so right, as we were saying the pipette aspiration assay exerts a pressure induces a tube of length l and the vesicle radius changes along with the tube length radius.

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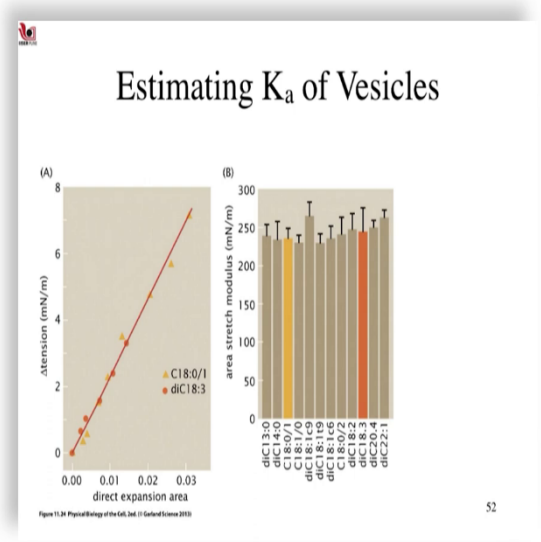


It turns out that as the direct expansion area increases, the tension also increases, or in other words increasing tension leads to the increased direct expansion area. And the slope of that allows us to estimate the area deformation modulus. These values, area stretch moduli of different vesicles made up of different lipids are indeed demonstrating here, that it is in the range of 250 milli Newtons per meter.

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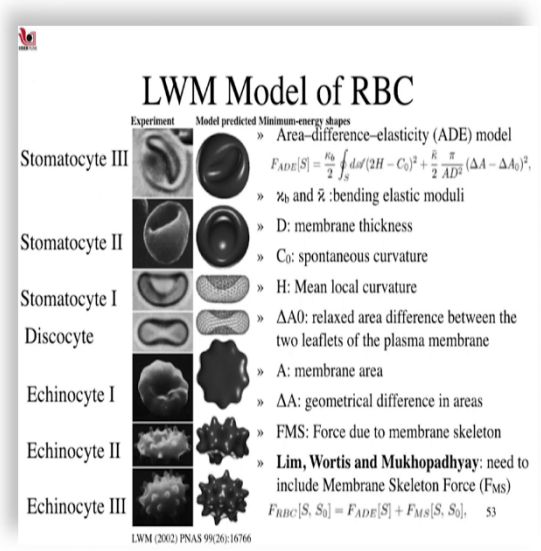


This brings us in a way to this idea that you can now combine all these terms and come up with a real membrane definition. And now, we always aim to try and get at whole cells, but the simplest cell we can start with is a cell that at least does not have a nucleus, it does not

have any very, very clear internal organelles, and does not undergo any gene expression changes to cell shell, cell shape response.

So, and that is the red blood cell, and this Lim, Wortis and Mukhopadhyay model has stood somehow the test of time, which essentially considers area difference in elasticity, and takes into consideration the bending modulus that is $\kappa_b$, the area change modulus that is $\bar{\kappa}$, kappa bar. And these combined with the area and the thickness of the membrane allow them to be able to simulate.

So in the left hand column of this image series, you see the experimental data, and the right-hand side of the simulation of this model, which when they included the membrane cytoskeleton force, which they called FMS, the membrane cytoskeleton, then gives them the full transition, which is due only to osmotic pressure from the disco sites, that is to say the normal red blood cells into these multiple shapes chromatocyte types, 1 and 2, 1, 2 and 3 equinocytes, that is star ship structures 1, 2 and 3 and a few others.

So, we would like to say that we are getting closer to an understanding of the membrane, but there are a lot of things more to be done, because a pure red blood cell membrane structure is not really a representation of what a true membrane might be like.

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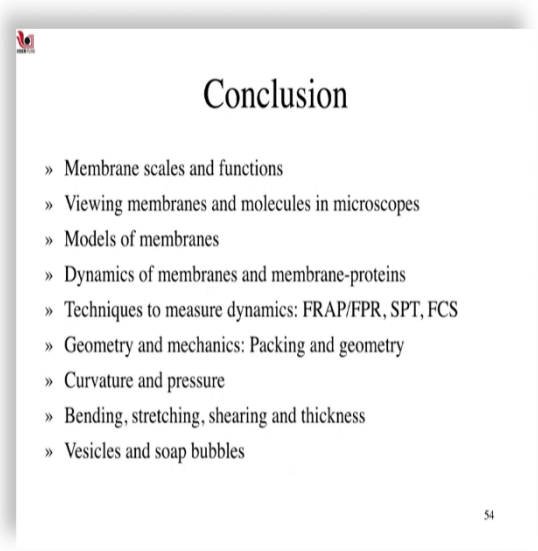


So, I am going to conclude that we have talked a little bit about membrane scales and functions viewing membranes from molecules to microscopes, models of membranes dynamics, how to measure them, we will talk about some of these methods. Packing

geometry, curvature and pressure, bending, stretching, shear, thickness, vesicles and soap bubble approximations.

And we will return to the measurement later, but for the moment, I am going to stop here, and we will continue next time with a slightly different topic, which relates to measurement but of something that we had not talked about which is related to a paper, and then move on to entropy and statistical mechanics. Thank you.